REMARKS

Entry of the foregoing amendments and reconsideration of the subject application as amended pursuant to and consistent with 37 C.F.R. § 1.111, in light of the remarks which follow, is respectfully requested.

Claims 1-10 are pending and under examination. Claims 1 and 9 have been amended. The amendment to claim 1 is supported in the specification, for instance, at page 14, lines 8-91. Claim 9 has been amended to correct antecedant basis. Claims 11-20, drawn to non-elected subject matter, have been canceled without prejudice to their being presented in a continuation application.

The specification has been amended to provide the complete address for the American Type Culture Collection and to properly format trademarks by capitalizing them.

These amendments do not introduce new matter.

Information Disclosure Statements

Applicants thank the Examiner for considering the references cited on the Information Disclosure Statements submitted April 26, 2004, July 17, 2006 and November 6, 2006 and for initialing and returning the corresponding Form 1449's.

Priority

In reviewing the priority claim, the Examiner states in part that this application is a continuation-in-part of application no. 09/894,644, filed Feb. 21, 2003. The cited application number is incorrect. As indicated on the first page of the specification as filed, the present application is a continuation-in-part of co-pending application no. 10/372,644, filed February 21, 2003.

We note however the filing date for 10/372,644 is incorrectly listed as June 21, 2003 in the declaration mailed on November 12, 2003. Accordingly, in accordance with 37 CFR §§1.76(c)(1) and (d)(1), a Supplemental Application Data Sheet is filed herewith to assure that the record reflects the correct priority claim.

Specification Objections

The Examiner has noted the use of trademarks in the application and objects to their not being capitalized The Examiner has also noted that the address for the American Type Culture Collection is incomplete. Applicants have therefore amended the specification to address both of these issues.

Rejection under 35 U.S.C. §112, 2nd paragraph

The Examiner has rejected claim 9 under 35 U.S.C. §112, 1st paragraph as allegedly vague and indefinite, for lacking proper antecedant basis for "the heavy chain antibody." Claim 9 has been amended to address this rejection. Accordingly, Applicants request the reconsideration and withdrawal of the rejection.

Rejections under 35 U.S.C. §103(a)

The Examiner has rejected claims 1-7 and 10 as being unpatentable over Jonker et al (WO 90/10701) in view of Pluenneke (US 2001/0021380).

The claims are drawn to the treatment of corneal transplant in a mammal comprising administering a composition *consisting essentially of* an antibody to tumor necrosis factor. It is Applicants' view that the cited references do not render obvious the amended claims for all the reasons that follow.

Jonker teaches that a combination of anti-TNF-alpha antibodies and anti-IFN-gamma antibodies is effective in organ transplant or graft rejection. Jonker explicitly teaches that administration of anti-TNF-alpha antibodies alone "has no detectable effect on the survival of the skin graft" (p. 3, 1st paragraph). Thus, Jonker teaches that only the combination of the two anti-cytokine agents is effective in treating transplanted organ graft.

Pluenneke teaches treating medical disorders characterized by abnormal or elevated expression of TNF-alpha by administering a soluble TNF-alpha antagonist. Several antagonists are disclosed, including antibodies to TNF-alpha. Pluenneke provides a laundry list of medical disorders thought to be characterized by abnormal or elevated expression of TNF-alpha. The list includes solid organ transplantation such as

transplantation of skin or cornea. Pluenneke, however, does not provide any data demonstrating that anti-TNF-alpha antibodies alone are efficacious in treating any solid organ transplanation, including corneal transplant. Thus, Pluenneke provides the skilled artisan little more than an invitation to try treating corneal transplant rejection by administering anti-TNF-alpha antibodies. However, at the time the present application was filed, it was believed in the art that corneal graft rejection was not characterized by elevated TNF-alpha protein. See Sano et al., 1998, IOVS 39:1953-1957 (copy attached), in particular p. 1955 and Table 2.

Thus, contrary to the Examiner's assertion, one of skill in the art would not reasonably expect that administration of anti-TNF-alpha antibodies alone could successfully treat corneal transplant rejection based on the combination of Jonker and Pluenneke. While Pluenneke suggests anti-TNF-alpha antibodies for treating rejection in corneal transplant, it is mere speculation. In addition, Pluenneke's speculation is contradicted by the knowledge in the art regarding TNF-alpha expression in corneal transplant rejection, specifically that TNF-alpha expression is abnormal or elevated in corneal transplant rejection. Moreover, Jonker provides experimental evidence that administration of an anti-TNF-alpha antibody alone *does not* treat rejection of organ transplant. In view of the actual data provided in Jonker and the knowledge in the art at the time of the present invention, therefore, the combination of Jonker and Pluenneke would not lead one of skill in the art to believe that corneal transplant rejection could be beneficially treated by anti-TNF-alpha antibodies alone.

Accordingly, the combination of Jonker and Pluenneke does not render obvious the claimed invention. Applicants request reconsideration and withdrawal of the rejection.

The Examiner has rejected claim 8 as being unpatentable over Jonker et al in view of Pluenneke and further in view of Skurkovich et al. (2003, Curr Opin Mol Ther 5:52-57) and Spinelli et al (1996, Nat Struc Biol 3:752-757).

Jonker and Pluenneke have been discussed above and those arguments stand with respect to this rejection of the claim.

Skurkovich et al. is a printed publication authored by the inventive entity of the present application. Thus, this reference is not "by others" as required under 35 U.S.C. §102(a). In addition, Skurkovich et al. was published in February 2003 (see attached PubMed abstract summary), less than a year before the actual filing date of the present application (August 5, 2003) and well past the earliest claimed priority date (June 5, 2001). Accordingly, Skurkovich et al. is not a valid reference available as prior art under either 35 U.S.C. §102(a) or (b). The Examiner may not rely on the teachings in this reference in the pending rejection. Applicants therefore do not further address this reference.

Spinelli fails to overcome the deficiencies of the combination of the teachings in Jonker and Pluenneke.

Spinelli discloses the crystal structure of a llama heavy chain variable domain. Spinelli is completely silent regarding corneal transplant rejection and TNF-alpha. The teachings in Spinelli do not overcome the negative teaching in Jonker that anti-TNF-alpha antibodies alone are not efficacious in treating organ transplant rejection or the knowledge in the art regarding TNF-alpha expression in corneal transplant rejection as disclosed in Sano et al. (1998). Thus, Spinelli cannot and does not suggest that treatment of corneal transplant rejection using anti-TNF-alpha antibodies would be successful. Hence, the combination of Jonker, Pluenneke and Spinelli do not render obvious the invention as claimed. Applicants respectfully request the reconsideration and withdrawal of this rejection.

CONCLUSION

In conclusion, this amendment and reply is believed to place the application in shape for allowance. Accordingly, the Examiner is respectfully requested to enter the Amendment and consider the arguments presented herein.

Respectfully submitted,

Boris Skurkovich et al.

Dated: February 26, 2007

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Attachment: Sano et al., 1998, "Cytokine Expression During Orthotopic Corneal Allograft Rejection in Mice" IOVS 39:1953-1957

Skurkovich et al., 2003, Curr Opin Mol Ther. 5:52-57 (PubMed abstract only)

1: Curr Opin Mol Ther. 2003 Feb;5(1):52-7.

Related Articles, Links

Anti-interferon-gamma antibodies in the treatment of autoimmune diseases.

Skurkovich B, Skurkovich S.

Brown Medical School, 169 Angell Street, Providence, RI 02912, USA.

Interferon (IFN)-gamma is an important immune regulator in normal immunity. When IFN gamma production is disturbed, various autoimmune diseases (ADs) can develop, in which we suggest that anti-IFN gamma could have a beneficial effect. Depending on the cell type in which IFN gamma synthesis is disturbed, different clinical manifestations may result. We have also proposed to remove tumor necrosis factor (TNF)-alpha, together with certain types of IFNs, to treat various ADs and AIDS, also an autoimmune condition. Anti-IFN gamma has been tested in several T-helper cell (Th1) ADs, including rheumatoid arthritis (RA), multiple sclerosis (MS), corneal transplant rejection, uveitis, Type I diabetes, schizophrenia (anti-IFN gamma and anti-TNF alpha), and various autoimmune skin diseases (alopecia areata, psoriasis vulgaris, vitiligo, pemphigus vulgaris and epidermolysis bullosa). A strong, sometimes striking, therapeutic response followed administration of anti-IFN gamma, indicating that it may be a promising therapy for Th1 ADs.

Publication Types:
- Case Reports
- Review

PMID: 12669471 [PubMed - indexed for MEDLINE]

and homozygous ACD patients is caused by the difference in age and accumulated layers of deposition.

Previously, corneal dystrophies have been classified by clinical and histopathologic findings. Therefore, differential diagnosis of corneal dystrophy is complicated. Our results suggest that in the future, corneal dystrophies should be classified according to their genotype.

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Cytokine Expression during Orthotopic Corneal Allograft Rejection in Mice

Yoichiro Sano, Hideya Osawa, Chie Sotozono, and Shigeru Kinoshita

PURPOSE. The acquisition of cell-mediated immunity against donor antigens has been shown to be associated with rejection of orthotopic corneal allografts, but the mechanisms that cause corneal allograft destruction in grafted tissue remain obscure. To determine which T-cell subsets infiltrate graft tissue and cause graft rejection, cytokine expression was examined in corneal tissue after orthotopic corneal allograft.

METHODS. BALB/c mice received orthotopic corneal allografts from either syngeneic BALB/c or allogeneic C57BL/6 donors. At 1 or 4 weeks after grafting, the mice were euthanatized, and their corneas were removed. Corneal tissue was frozen, homogenized, and placed in phosphate-buffered saline (PBS). Each sample consisted of five corneas in 500 ml PBS. After centrifugation, the supernatant was collected, and the concentration of the following cytokines was measured by enzyme-linked immunosorbent assay: interleukin (IL)-1α, IL-2, IL-4, IL-10, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α.

RESULTS. Significantly increased amounts of proinflammatory cytokines (IL-1α and TNF-α) were detected in supernatants from all grafted corneas (both syngeneic and allogeneic) at 1 week after grafting. At 4 weeks after grafting, supernatants from normal corneas, corneas with syngeneic grafts, and corneas with accepted corneal allografts contained undetectable amounts of IL-2 and IFN-γ, whereas supernatants from corneas with rejected corneal allografts contained significant amounts of IL-2 and IFN-γ. There were no significant differences in the amounts of IL-4 or IL-10 among all samples. Histologic examination confirmed the expression of IL-2 and IFN-γ in rejected corneal allografts.

CONCLUSIONS. Because IL-2 and IFN-γ are secreted primarily by T-helper type 1 (Th 1) cells, whereas IL-4 and IL-10 are secreted by T-helper type 2 (Th 2) cells, these results indicate that Th 1-type cytokines, rather than Th 2-type cytokines, contribute to the rejection of orthotopic corneal allografts in graft tissue. (*Invest Ophthalmol Vis Sci.* 1998;39:1953-1957)

Study of the immune response in orthotopic corneal transplantation has revealed unique features associated with the anterior segment of the eye, compared with other types of organ transplantation. Unlike other types of organ transplants, corneal grafts that display major histocompatibility complex class I or class II alloantigens only are less likely to be rejected, whereas grafts that display only minor transplantation antigens are rejected much more frequently.[1,2] It has been shown also that the acceptance of orthotopic murine corneal allografts correlates positively with the development of donor-specific anterior chamber-associated immune deviation.[3] Moreover, recent studies have reported that donor-specific delayed hypersensitivity responses detected after orthotopic corneal allografts are directed at donor-minor alloantigens, but not at major histocompatibility complex alloantigens.[4]

Using experimental animals, Callanan et al[5] performed a study to characterize the mechanisms of orthotopic corneal allograft rejection and reported a correlation between rejection and cell-mediated immunity in the recipients. Although this study revealed the acquisition of systemic cell-mediated immu-

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nity against donor antigens, whether these immune responses are elicited in the local environment of the eye during orthotopic corneal allograft rejection remains uncertain.

In this study we examined the immune response during orthotopic corneal allograft rejection by quantifying the cytokines in grafted tissue. We report that significant amounts of interleukin (IL)-2 and interferon (IFN)-γ, but not IL-4 and IL-10, were detected only in rejected corneal tissue. Our results suggest a correlation between T-helper type 1 (Th 1) cytokines and orthotopic corneal allograft rejection.

Materials and Methods

Mice

Six- to 8-week-old BALB/c and C57BL/6 mice were purchased from Shimizu Laboratory Supplies (Kyoto, Japan). All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Corneal Transplantation and Grafting

Orthotopic corneal transplantation was performed as described previously.[1] Briefly, donor central corneas (2 mm in diameter) were excised using Vannas scissors and placed in chilled phosphate-buffered saline (PBS). Animals were anesthetized with intraperitoneal injections of ketamine (3–4 mg/animal) and xylazine (0.1 mg/animal). The graft bed was prepared by excising with Vannas scissors a 2-mm site in the central cornea of the right eye. The donor cornea was then placed in the recipient bed and secured with eight interrupted sutures (11-0 nylon). All grafted eyes were examined after 72 hours; at that time, grafts with technical difficulties (hyphema, infection, or loss of the anterior chamber) were excluded from further consideration. At 9 days after grafting, all sutures were removed.

Evaluation and Scoring of Orthotopic Corneal Transplants

After corneal transplantation, grafts were examined by slit lamp microscopy at weekly intervals. Each time, grafts were scored for opacity and neovascularization. A scoring system was devised to describe in semiquantitative terms the extent of opacity (0–5+), as described previously.[1] Grafts with opacity scores of 2+ or greater at 8 weeks were considered to have been rejected.

Cytokine Quantitation

The cytokines in corneal tissues were quantified as described previously.[6] Corneas (4 mm in diameter) were excised from the limbus at 1 or 4 weeks after grafting. Each test sample was comprised of five corneas. The samples were frozen with liquid nitrogen and then homogenized in chilled PBS at a ratio of 100 μl per cornea. The supernatants were collected by centrifugation at 1500*g* for 10 minutes and stored at −80°C until used. Cytokine production levels were measured using enzyme-linked immunosorbent assay systems for IL-1α, tumor necrosis factor (TNF)-α (Genzyme, Cambridge, MA), IL-2, IL-4, IL-10, and IFN-γ (Endogen, Boston, MA). The limits of detection were 15 pg/ml for IL-1α and TNF-α, 3 pg/ml for IL-2, 5 pg/ml for IL-4, 0.14 U/ml for IL-10, and 15 pg/ml for IFN-γ. In all enzyme-linked immunosorbent assay experiments, recombinant murine cytokines were used as positive controls. At least three samples were tested in each group, and statistical analysis was performed on their values using the Student's *t*-test.

Immunohistochemical Analysis

Based on the results of the enzyme-linked immunosorbent assays, immunohistochemical analyses were performed to determine the expression of cytokines in the tissue. Corneas obtained 4 weeks after grafting were fixed with 4% paraformaldehyde in 0.1 M PBS and then dehydrated with 20% sucrose in 0.1 M PBS. After embedding of the corneas in OCT compounds (Miles, Elkhart, IN), 7-μm cryostat sections were prepared, air-dried, and then stored at −80°C. Sections were treated with 0.5% H_2O_2 for 60 minutes at room temperature and incubated with 20 μg/ml goat polyclonal antibody to IL-1α (R&D Systems, Minneapolis, MN) or rat monoclonal antibody to IL-2 or IFN-γ (Endogen, Boston, MA) for 16 hours at 4°C, incubated further with biotinylated anti-goat or anti-rat IgG (Vector Laboratories, Burlingame, CA) for 2 hours at room temperature, and then incubated in avidin-biotin-peroxidase complex (ABC; Vector Laboratories; 1:100) for 90 minutes at room temperature. To detect immune complexes, the sections were immersed for 10 minutes at room temperature in 0.02% 3, 3′-diaminobenzidine in 100 ml of 0.05 M Tris-HCl buffer (pH 7.6) containing 20 μl of 30% H_2O_2. Between each step, the sections were thoroughly washed in 0.1 M PBS. Corneal sections were also stained with hematoxylin and eosin. In all assays, negative controls were prepared using 20 μg/ml goat or rat IgG for the primary antibody.

Results

Appearance of Orthotopic Corneal Allografts and Isografts

Ten BALB/c mice received orthotopic corneal isografts from BALB/c donors, and 10 BALB/c mice received orthotopic corneal allografts from C57BL/6 mice. As previously reported,[1] all grafts, isografts and allografts, showed slight edema and opacity at 1 week after grafting; however, all grafts appeared to be clear at 2 weeks after grafting. At 4 weeks after grafting, all isografts appeared completely clear. Five corneal allografts of 10 (50%) developed moderate to severe opacity approximately 4 weeks after grafting and were considered to have been rejected, whereas the other 5 corneal allografts (50%) remained clear and were considered to have been accepted.

Proinflammatory Cytokine Production in Corneal Tissue after Grafting

Because IL-1α and TNF-α are known as proinflammatory cytokines, which cause nonimmune-mediated inflammatory response, the secretion of proinflammatory cytokines was studied at 1 week after grafting, when all grafts (isografts and allografts) showed mild edema and opacity, and at 4 weeks, when some of the allografts had been rejected. The supernatants from normal BALB/c corneas contained 4.0 ± 0.5 (mean ± SEM) pg/cornea of IL-1α and 8.9 ± 0.9 pg/cornea of TNF-α. The supernatants from corneal tissue with isografts contained significantly increased amounts of IL-1α (7.5 ± 0.3 pg/cornea; $P = 0.04$) and TNF-α (15.2 ± 2.4 pg/cornea; $P = 0.04$) as did those from corneal tissue with allografts (5.6 ± 0.3 pg/cornea, $P = 0.02$, and 13.5 ± 1.4 pg/cornea, $P = 0.02$, respectively) at 1 week after grafting. IL-1α and TNF-α were

TABLE 1. Cytokine Concentrations Found in Corneal Tissue at 1 Week after Grafting

	Proinflammatory		Th 1		Th 2	
	IL-1α (pg/cornea)	TNF-α (pg/cornea)	IL-2 (pg/cornea)	IFN-γ (pg/cornea)	IL-4 (pg/cornea)	IL-10 (U/cornea)
Normal cornea†	4.0 ± 0.5	8.9 ± 0.9	ND	ND	4.6 ± 0.6	ND
Cornea with isograft‡	7.5 ± 0.3*	15.2 ± 2.4*	ND	ND	5.0 ± 0.3	ND
Cornea with allograft§	7.1 ± 0.7*	13.5 ± 1.4*	ND	ND	6.0 ± 0.4	ND

Th 1/Th 2, T-helper type 1 or type 2 cells, respectively; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; ND, not detected.
* Mean value is significantly greater than normal cornea.
† Normal corneas were obtained from naive BALB/c mice.
‡ Corneas obtained from BALB/c mice with syngeneic BALB/c corneal grafts.
§ Corneas obtained from BALB/c mice with allogeneic C57BL/6 corneal grafts.

also quantified at 4 weeks after grafting. Significantly increased amounts of IL-1α were detected only in supernatants from corneal tissue with rejected allografts ($P = 0.02$). No increase in the amount of TNF-α was detected in any of the samples (Table 1).

Th 1 and Th 2 Cytokine Production in Corneal Tissue after Grafting

Because Th 1 cells secrete IL-2 and IFN-γ, whereas T-helper type 2 (Th 2) cells secrete IL-4 and IL-10, the amounts of these cytokines were quantified in corneal tissue to examine which immune response predominates during corneal allograft rejection. IL-2, IFN-γ, and IL-10 were not detected in the supernatants from normal corneal tissue. The supernatants from corneal tissue with either isografts or allografts at 1 week after grafting contained undetectable amounts of IL-2, IFN-γ, and IL-10. However, IL-4 was detected in the supernatants from corneal tissue with isografts (5.0 ± 0.3 pg/cornea) and allografts (6.0 ± 0.4 pg/cornea), but the amounts were not significantly different from those in normal BALB/c corneal tissue (4.6 ± 0.6 pg/cornea) (Table 2).

At 4 weeks after grafting, significantly increased amounts of IL-2 (16.4 ± 4.5 pg/cornea; $P = 0.01$) and IFN-γ (12.0 ± 0.3 pg/cornea; $P = 0.001$) were detected in the supernatants of corneal tissue with rejected allografts, whereas supernatants from corneal tissue with either isografts or accepted allografts contained undetectable amounts of IL-2 and IFN-γ. IL-10 was not detected in any of the supernatants. IL-4 was detected in supernatants from corneal tissue with isografts (5.9 ± 0.4 pg/cornea), accepted allografts (4.7 ± 1.0 pg/cornea), and rejected allografts (4.8 ± 0.8 pg/cornea), but levels were not significantly different from those in normal BALB/c corneas (Table 2).

Immunohistochemical Analysis

Because significant amounts of IL-2, IFN-γ, and IL-1α were detected, subsequent experiments were designed to examine the origin of these cytokines in rejected corneal allografts. The grafted tissues were stained with anti-IL-2, anti-IFN-γ, and anti-IL-1α antibody using avidin-biotin-peroxidase complex assays. In the rejected corneal tissues, strongly positive staining for IL-2 and IFN-γ was observed in the stroma (Fig. 1A, 1B). Positive staining for IL-1α was also found in the epithelium and stroma of rejected tissue, whereas weakly positive staining was found only in the epithelium of accepted tissue (Figs. 1C, 1D).

DISCUSSION

Previous reports in which laboratory animals were used have indicated that cell-mediated immunity plays a significant role in corneal allograft rejection. Using rat corneal transplantation models, Callanan et al.[5] have shown that rejection of ortho-

TABLE 2. Cytokine Concentrations Found in Corneal Tissue at 4 Weeks after Grafting

	Proinflammatory		Th 1		Th 2	
	IL-1α (pg/cornea)	TNF-α (pg/cornea)	IL-2 (pg/cornea)	IFN-γ (pg/cornea)	IL-4 (pg/cornea)	IL-10 (U/cornea)
Normal cornea†	4.0 ± 0.5	8.9 ± 0.9	ND	ND	4.6 ± 0.6	ND
Cornea with isograft‡	6.0 ± 0.9	8.1 ± 0.8	ND	ND	5.9 ± 0.4	ND
Cornea with accepted allograft§	5.2 ± 0.4	10.3 ± 0.9	ND	ND	4.7 ± 1.0	ND
Cornea with rejected allograft‖	10.5 ± 0.7*	9.8 ± 1.1	16.4 ± 4.5*	12.0 ± 0.3*	4.8 ± 0.8	ND

Th 1/Th 2, T-helper type 1 or type 2 cells, respectively; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; ND, not detected.
* Mean value is significantly greater than normal cornea.
† Normal corneas were obtained from naive BALB/c mice.
‡ Corneas obtained from BALB/c mice with syngeneic BALB/c corneal grafts.
§ Corneas obtained from BALB/c mice with accepted allogeneic C57BL/6 corneal grafts.
‖ Corneas obtained from BALB/c mice with rejected allogeneic C57BL/6 corneal grafts.

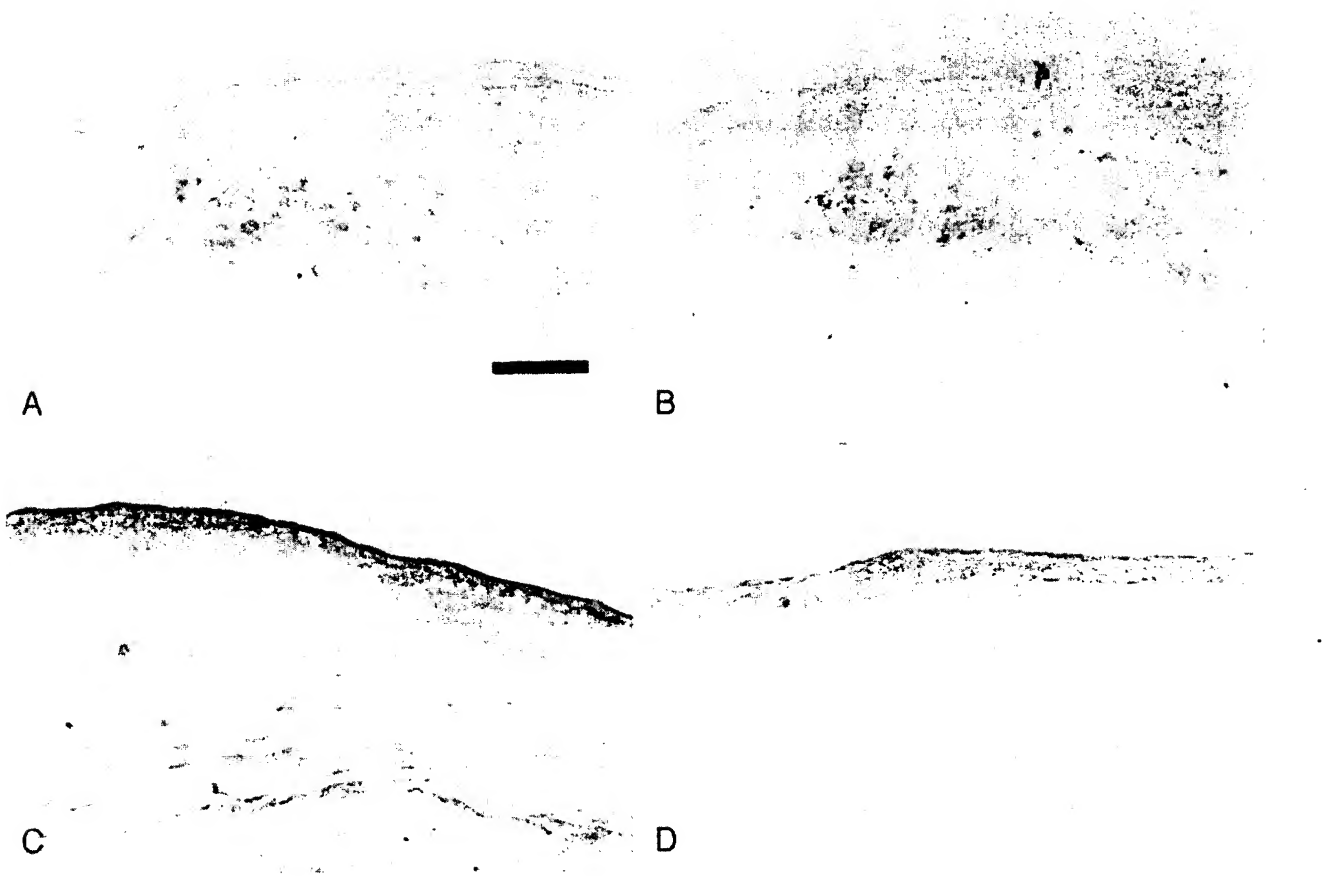

FIGURE 1. Immunohistochemical staining of corneal allografts at 4 weeks after grafting. Interleukin (IL)-2-positive cells (A) and interferon (IFN)-γ-positive cells (B) are present in the rejected corneal allograft. Strong staining for IL-1α can be seen in the epithelium and stroma of rejected corneal tissue (C), whereas IL-1α staining is slight in the epithelium of accepted corneal tissue (D). Bar, 100 μm.

topic corneal allografts is mediated primarily by cytotoxic T cells. Data from Joo et al[7] indicated that donor-specific DH, rather than CTL, is associated with corneal allograft rejection in mice. Although these reports suggest a correlation between corneal allograft rejection and systemic donor-specific cell-mediated immunity, the mechanisms of graft rejection remain uncertain in corneal tissue. It has been suggested that the anterior segment of the eye, including the cornea itself, is an immunologically privileged site. Cousins et al[8] have shown that intraocular DH was suppressed when *Mycobacterium tuberculosis* antigens were injected into the anterior chamber of the eyes of mice that had been systemically immunized with *M. tuberculosis* antigens previously. Ksander and Streilein[9] have shown that cytotoxic T cells directed at the alloantigens of intraocular tumors could not fully differentiate in the eye, although precursors of these T cells could be detected in the draining lymph nodes. Therefore, even though recipients with corneal allografts acquire donor-specific delayed hypersensitivity or cytotoxic T lymphocytes, the local microenvironment of the eye may suppress these immune responses at the local site.

T cells can be divided into two subpopulations on the basis of the expression of surface markers. T cells expressing CD4 molecules are mostly helper T cells that secrete cytokines and "help" the other immune cells to differentiate and function. T cells expressing CD8 molecules are mostly cytotoxic T cells that kill the target cells. Helper T cells can be classified into subsets that produce different sets of cytokines on activation. Th 1 cells produce IL-2, IFN-γ, and TNF-α but not IL-4 or IL-5, and predominate in cell-mediated immune responses, whereas Th 2 cells produce IL-4, IL-5, and IL-10, but not IL-2 or IFN-γ, and play a prominent role in humoral immunity. The present study has shown that the expression of IL-2 and IFN-γ is associated with graft rejection in corneal tissue. The results of immunohistochemical analysis indicate that the expression of these cytokines seems to have originated from the infiltrating cells in the rejected tissue. CD4 Th 1 cells and CD8 cytotoxic T cells are able to produce these cytokines; therefore, it is likely that these cells actually infiltrate into the donor graft tissue and cause graft destruction, which results in graft failure. He et al.[10] reported that systemic anti-CD4 antibody administration to recipients promoted corneal allograft survival, whereas anti-CD8 antibody administration did not. They concluded that CD4-positive T cells, rather than CD8-positive T cells, play an important role in graft rejection. Therefore, infiltrating cells that secrete IL-2 and IFN-γ as detected in our studies may be CD4 T cells, which cause direct cytotoxicity or mediate a DH response in graft tissue.

The importance of the increases in IL-1α and TNF-α early after grafting is also noteworthy. Because these proinflammatory cytokines were detected in syngeneic and allogeneic grafted tissues at 1 week after grafting, these cytokines seemed to be secreted in response to the grafting procedure. Because IL-1 has been known to induce Langerhans cell migration into the central cornea,[11] which is considered to be important for the recognition of alloantigens in grafted tissue, the increase in these proinflammatory cytokines early after grafting may con-

tribute to graft rejection. In fact, Dana et al.[12] have reported that the topical administration of IL-1 receptor antagonist after grafting promotes corneal allograft survival.

A recent study reported by Torres et al.[13] foundincreased levels of proinflammatory cytokine mRNA up to 7 days postoperatively when both autografts and allografts showed mild to moderate opacity and revealed more expression of Th 1 cytokine mRNA than Th 2 cytokine mRNA in rejected allograft corneal tissue. Our results agree with this report and have demonstrated the increased expression of cytokine mRNA that was detected in their experiments, resulting in increased cytokine protein levels.

In summary, we have reported cytokine expression patterns after orthotopic corneal transplantation in grafted tissue. Our results showed that an increase in proinflammatory cytokine (IL-1α and TNF-α) expression was detected in syngeneic and allogeneic graft tissue and that the production of IL-2 and IFN-γ was detected only in rejected corneal allografts. These results suggest the importance of proinflammatory cytokines for the recognition of donor alloantigens in grafted tissue and the role of Th 1 cytokines as effectors of corneal allograft rejection in corneal tissue. We suggest that the inhibition of proinflammatory cytokines early after grafting or the inhibition of Th 1 cytokines may contribute to the prolongation of corneal allograft survival.

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Decorin and Biglycan of Normal and Pathologic Human Corneas

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Purpose. Corneas with scars and certain chronic pathologic conditions contain highly sulfated dermatan sulfate, but little is known of the core proteins that carry these atypical glycosaminoglycans. In this study the proteoglycan proteins attached to dermatan sulfate in normal and pathologic human corneas were examined to identify primary genes involved in the pathobiology of corneal scarring.

Methods. Proteoglycans from human corneas with chronic edema, bullous keratopathy, and keratoconus and from normal corneas were analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), quantitative immunoblotting, and immunohistology with peptide antibodies to decorin and biglycan.

Results. Proteoglycans from pathologic corneas exhibit increased size heterogeneity and binding of the cationic dye alcian blue compared with those in normal corneas. Decorin and biglycan extracted from normal and diseased corneas exhibited similar molecular size distribution patterns. In approximately half of the pathologic corneas, the level of biglycan was elevated an average of seven times above normal, and decorin was elevated approximately three times above normal. The increases were associated with highly charged molecular forms of decorin and biglycan, indicating modification of the proteins with dermatan sulfate chains of increased sulfation. Immunostaining of corneal sections showed an

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